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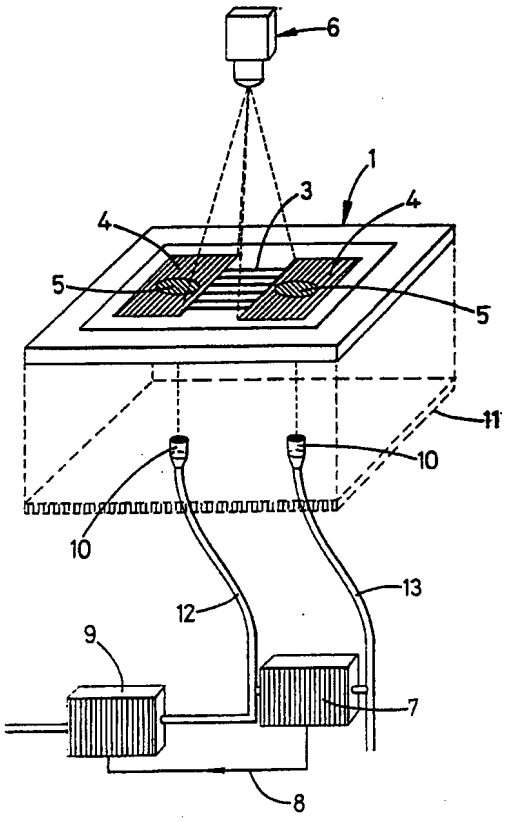
(57) Abstract

A method of performing biorheological measurements on a suspension (4) of cells by causing some of the cells to pass through one or more microsize tunnels (3) in a solid medium (1, 2) under a pressure difference between the tunnel ends and monitoring (6) their passage through the tunnel or tunnels, characterised in that the pressure difference is monitored to produce a signal which is recorded or is utilised to provide feedback to regulate the biorheological measurements. Apparatus for performing this method may have such a pressure feedback control, or may have tunnels with at least partly transparent walls for transmitting optical information to a detector for the biorheological measurements.



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Biorheological Measurement

This invention relates to the measurement of biological cell rheological properties and to apparatus for use in such measurement.

The rheology of biological cells is of considerable interest to workers in the medical and pharmaceutical fields. Of particular interest is the sub-discipline of microrheology. This concerns inter alia the flow of cells in channels of dimensions approaching those of the cells themselves. One example of this is the flow of human red and white blood cells in the capillary networks of the human body. In these vessels cells transit serially whilst undergoing deformation due to the capillary diameter being less than the cell diameter. The degree of the cells' deformability is related to the elastic constants of the cells which are, in turn, related to biochemical properties of the cells. It is suggested that some disease states can affect the biochemistry and hence deformability of the cell, and in doing so symptoms of circulatory disease result. For example, the disease "Diabetes Mellitus" can result in progressive circulatory dysfunction; this is believed to be contributed to by a change in erythrocyte cellular deformability.

Due to the extreme difficulty of in-vivo measurement of these properties recourse is made to in-vitro techniques. Unfortunately the rheological properties of cell suspensions make it impossible to determine accurately microrheological characteristics from macrorheological measurements. In an attempt to overcome this, several techniques have been

developed to measure cells passing through fabricated microchannels. Potentially the most accurate of these existing techniques is to pass cells individually through glass micropipettes whilst measuring their flow properties.

Micropipettes have offered the most physiologically analogous technique, but several problems have restricted their application. These fall into two groups. Firstly, fabrication and utilisation related factors limit measurement accuracy. Secondly, due to several factors the number of cells of one sample that can be measured is of insufficient statistical significance for detailed analysis. The latter limitation precludes the use of this technique, in its current implementation, for the identification of small sub-populations of cells with aberrant rheological properties.

Macroscopic filtration techniques have been recognised as an alternative, and have been developed in parallel, but there have been problems of reproducibility of results, due to the differences in diameter between different pores of the same membrane and between different membranes. Some refined forms of filtrometer: the Single Erythrocyte Rigidometer: SER and its lineal development, the Cell Transit Analyser: CTA, overcome the intra analysis reproducibility problem by means of a continuous flow technique through one or more pores in a membrane. Furthermore, operator effects are virtually removed by automating the erythrocyte transit measurement. We categorize the CTA as a Multi Channel Non Concurrent Transit device: MCNCT. The CTA and SER do, however, suffer from

the drawback that it is impossible in current devices to differentiate between the steady state flow of an erythrocyte within a pore in the membrane and entrance effects as the erythrocyte initially deforms to enter the pore: only a global 'occlusion time' is measured. This drawback is related to the difficulty of fabricating a device of this form that offers a means of finely monitoring the cells' "velocity profile" during the "transit" as distinct from measuring the global "occlusion time".

A further disadvantage of MCNCT devices is that they do not offer a significantly higher cell throughput than an SER. This is due to the potential ambiguities that would result from concurrent transits being monitored as a composite signal. As a result only one pore of the MCNCT can contain a cell at given time. This is achieved by using a low haematocrit and attempting to reject occasional multiple transits by an appropriate hardware or software algorithm. The main advantage of MCNCT devices is that they are tolerant of clogging, rather than suddenly ceasing measurement as is the case in a micropipette or SER.

A recently-developed CTA is described by Koutsouris D., Guillet R., Lelivre J.C., Guillemin M.T., Beuzard Y. 1986: Mechanical properties of the erythrocytes indicated by cell transit time analysis. 6th International Congress of Biorheology, Vancouver 1986.

The object of the present invention is to overcome the aforesaid problems. Accordingly, the invention provides a method of performing biorheological measurements as defined in Claim 1. The tunnel(s) may be formed in the

substrate to a high precision utilising a micromachining technique in conjunction with other processes.

Advantageously, a pair of reservoirs for the suspension of cells is formed in the solid medium, the or each of said tunnels extending between the reservoirs.

The location or speed of transit of cells in the tunnel(s) at any instant may be recorded by imaging or by direct measurement by transducers or sensors such as charge sensors, integral with or adjacent to the solid medium. With a knowledge of the tunnel dimensions, rheological parameters may be calculated.

The invention also provides apparatus for performing biorheological measurements, as defined separately from different aspects in Claims 6 and 7.

It will be appreciated that the invention combines the facility for automation and the physical parallelism of the CTA with the observability of the micropipette. In addition, the possibility of introducing constrictions in the tunnels, impossible with pores in a filter membrane, now exists and will enable new types of measurement to be made. These properties may be achieved for example by implementing a planar array of high precision capillaries as a microfabricated silicon flow cell. The design and fabrication techniques employed in such a capillary array overcome many of the difficulties associated with micropipettes. We shall call the class of devices to which such a flow cell belongs as "Multi-Channel Multiple Concurrent Transit": MCMCT.

In making the aforesaid measurements, a pump may be

required to create the pressure difference to move the cells along the tunnel(s). Such a pump has to be capable of pumping a defined, small, quantity of fluid in a manner free of discontinuities.

Delivery of very low volumes of fluid in a time-continuous manner is a recognised problem in science, medicine and technology. An example of a conventional approach is the use of stepper motor driven syringes. This technique, by its nature, results in a microscopically discontinuous pumping action. Furthermore stiction effects between the syringe bore and plunger seal can result in further discontinuities.

In another aspect the present invention relates to such a pump, and is defined in Claim 11. In use the reservoir is pre-charged with the fluid to be pumped, and the piezoelectrically-generated force is applied; as a result the fluid is pumped from the reservoir, for example, via an orifice which may be provided at an appropriate location. The use of a piezoelectric element yields a continuous input/output function. In addition, with appropriate implementation high rates of change of flow rate may be achieved, for rapid response to the feedback pressure-control signal in apparatus for rheological measurements embodying the present invention, or for other purposes.

The invention will now be further described by way of example with reference to the accompanying schematic drawings, not drawn to scale, in which:

Figure 1 is an exploded perspective view of one form

of apparatus for carrying out measurements in accordance with the invention;

Figure 2 is a sectional view of part of the apparatus of Figure 1, in a modified form, and to a larger scale;

Figure 3 is a partly exploded perspective view of a measuring system utilising the apparatus of Figures 1 and 2;

Figure 4 is a diagram of a single tunnel, showing different stages of the passage of a cell along the tunnel; and

Figure 5 is an exploded perspective view of a piezoelectric pump which can be used as one of the elements in the system of Figure 3.

Referring now to the drawings, in which corresponding parts are denoted by identical reference numerals, the apparatus shown in Figure 1 comprises a silicon substrate 1 and a thin glass cover slip 2. A number of equal-length parallel channels 3 with square base edges and constant cross-section and of appropriate dimensions are chemically etched into the upper surface (as seen in Figure 1) of the substrate 1. The parallel channels terminate in reservoir structures 4 also etched into the upper surface of the substrate 1. Outlet holes 5 connect the centre of the reservoir structures 4 with the lower surface (as seen in Figure 1) of the substrate. Typical dimensions for the substrate would be 5 mm long by 4 mm wide and the length of the channels 3 could typically be 100μm.

The thin cover slip 2 is placed over the channels 3 and reservoirs 4 so that the channels 3 in effect become

tunnels. The cover slip 2 is pressed into intimate contact with the substrate 1 by gas pressure. Alternatively it could be permanently sealed to the substrate 1. The channels 3, reservoirs 4 and holes 5 thus form a continuous chamber closed except for the orifices on the lower surface of the substrate provided by the holes 5.

The modified apparatus of Figure 2 is similar in most respects to that of Figure 1, the measurement cell fabricated using the emerging technology of silicon micromachining. The device consists of two layers of silicon substrates 1,2 bonded together.

Fabrication is commenced on the lower element 1 which has an array of channels 3 chemically etched into the surface. Channels have been made in a range of cross-sectional dimension options, however 5μm x 5μm has been used for preliminary evaluation. These dimensions are to sub-micron tolerances, and presently have an intra device accuracy of $<$ 100nm and an inter device accuracy of $<$ 200nm. The cross-sectional profile of the first prototypes is square with filleted bottom corners, but it will have a curved profile, e.g. cylindrical, in future development. The channels are 100μm in length. These dimensions have been chosen to mimic a typical physiological capillary in body tissue. The array of channels is terminated at both ends with a reservoir structure 4,4 of approximately 15μm depth. In the centre of each reservoir 4, a deep shaft 5 has been etched through to the lowermost surface of the silicon.

The upper layer 2 of silicon, which replaces the glass cover slip of Figure 1, is prepared by chemically

growing a layer 202 of transparent silicon dioxide on the surface of a silicon substrate 201. Then an opening 203 in the form of a shaft is etched to the interface between the silicon dioxide 202 and silicon 201 of the upper layer. The etchant used for this etch does not react with silicon dioxide and hence yields an Si02 "window" in a Si "frame". The window is dimensioned and positioned to yield a view of the channels 3 when the two elements 1,2 of silicon are combined.

The final stage of preparation involves the alignment and atomic bonding of the adjacent, clean, surfaces of the two layers. In this process the channels are converted to capillaries with transparent faces formed by the window.

The resulting flow cell measures approximately 4 x 5 mm and is precisely mounted on a carrier 11 complete with two capillary feed tubes 12,13 as shown in Figure 3.

In use of the apparatus of Figures 1 and 2, red blood cells (in this example only) in suspension in the reservoir 4 are introduced into tunnel openings in a random orientation, undergoing deformation, and are fed along the tunnels formed by the channels 3, a process which may take 2-3 seconds. The cell positions in the tunnels are monitored by optical imaging via a video camera 6 as shown in Figure 3. The capillary array is imaged via an incident illumination (metallurgical) microscope. In order to optimise the contrast ratio between the cell and channel, 400 nm illumination in the far visible violet is used. This wavelength is absorbed by the haemoglobin in erythrocytes.

As a result the erythrocytes are contrasted as dark objects against the highly reflective light background of the silicon. The resulting image is converted to an analogue electrical signal by the video camera 6 and subsequently digitised by a 256 x 256 pixel video digitiser. The resulting data is fed to a computer for image processing. The output of the camera is processed such that the temporally coincident movements of cells in separate tunnels can be monitored concurrently, and so that any tunnels that are, or have been, blocked are ignored. The windows 204 may extend over a major part of the tunnel length, or over its entire length as shown.

A differential pressure sensor 7 provides a signal that is used in a control loop 8 to regulate the flow rate of fluid via a flow regulating device 9 in order to maintain a constant pressure differential between the inlets and outlets of the tunnels. In this manner, interaction between cells in separate tunnels due to the flow rate differences between the tunnels induced by the presence or absence of cells is eliminated. The deformability of cells is calculated utilising the spatial information from the camera, the time reference from within the associated instrumentation, the predetermined tunnel geometry, and the constant regulated pressure differential across the tunnels. The pressure difference is typically in the physiological range of (2-20) mm H_2O; accuracy over this range is achievable using a discrete silicon diaphragm differential, or absolute, fluid pressure transducer.

Figure 4 shows a blood cell 400 entering a channel 3

under the pressure differential, slowly deforming as it does so. The reverse process of expansion occurs on exit. Consequent variation of its speed at different stages may be monitored, as may its actual volume be determined. In alternative forms of channels, there may be a variation of cross-section along its length, such as an intermediate constriction.

One method of introducing fluid i.e. a cell suspension to the apparatus is illustrated in Fig. 3. In this implementation the substrate 1 forms a fluid tight seal with a support plate 11 (shown in dotted line, separated from the substrate) through which holes 10 have been formed. The holes 10 are positioned so that they align with the holes 5 in the substrate. The thickness of the plate 11 is such that it enables the connection of capillary pipes or tubing 12 and 13 to the holes 10 through appropriate connectors.

One advantageous form for the flow regulating device 9 is the piezoelectric pump shown in Figure 5. This pump comprises a rigid cylindrical casing 21 with a closed lower end (in the orientation of Figure 5). A deformable reservoir 22 is contained within the casing and this has inlet and outlet tubes 23 and 24 which extend through diametrically opposite holes 25 in the casing 21. A disc 26 of piezoelectric material fits on top of the reservoir 22 and within the casing 21. A spacer 27 fits over the disc 26 and within the casing 1. The upper end of the casing 21 is closed by a cover plate 28 which is secured in position by bolts 29 to maintain the components in the casing 21 in

correct alignment. The disc 26 is provided with an electrical control voltage through electrical leads 30 passing through the wall of the casing 21.

In operation the reservoir 22 is charged via a valve 31 which allows fluid to enter from inlet tube 32. While the reservoir 22 is being charged no deforming force is applied by the piezoelectric disc 26. When charging is complete the valve 31 is closed and a voltage is applied to the piezoelectric disc resulting in its extension and in deformation of the reservoir 22, hence pumping.

The reservoir 22, being a separate self-contained item, can be supplied as a disposable item suitable for use with hazardous materials or materials where interpump cycle contamination cannot be tolerated. The reservoir 22 may be formed as a flat spiral of deformable tubing.

Use of a spacer plate of greater area than the piezoelectric disc disposed between the disc and the reservoir will increase the area over which the piezoelectric force acts. Hence the pumped volume for a given piezoelectric disc might be increased to a limit determined by other parameters.

CLAIMS

1. A method of performing biorheological measurements on a suspension (4) of cells by causing some of the cells to pass through one or more microsize tunnels (3) in a solid medium (1,2) under a pressure difference between the tunnel ends and monitoring (6) their passage through the tunnel or tunnels,

characterised in that the pressure difference is monitored to produce a signal which is recorded or is utilised to provide feedback to regulate the biorheological measurements.

2. A method according to claim 1, in which the pressure difference signal is utilised to provide feedback to control the pressure difference such as to maintain it constant.

3. A method according to claim 1 or claim 2, comprising observing the passage of successive cells through the or each tunnel (3) using a detector (6) effective over at least a major part of the length of the tunnel.

4. A method according to claim 3, comprising determining the velocity of motion of the cell (400, Fig.4) at two or more stages of its passage into, through and out of the tunnel (3).

5. A method according to claim 3, in which the cells from a common reservoir (4) are drawn into several parallel microsize tunnels (3), all under the same pressure difference.

6. Apparatus for performing biorheological measurements on a suspension of cells, comprising a solid medium (1,2) having therein one or more microsize tunnels (3)

communicating with a reservoir (4) for the cell suspension, and means (9) for applying a pressure difference between the ends of the or each tunnel, characterised in that at least one part (204) of the wall of the or each tunnel (3) is transparent to at least a range of optical wavelengths, and for at least a major part of the length of the tunnel, and that the apparatus comprises detector means (6) associated with the or each tunnel responsive to optical information transmitted through the transparent part from within the tunnel, for observing the passage of the cells.

7. Apparatus for performing biorheological measurements on a suspension of cells, comprising a solid medium (1,2) having therein one or more microsize tunnels (3) communicating with a reservoir (4) for the cell suspension, and means (9) for applying a pressure difference between the ends of the or each tunnel, characterised in that the apparatus comprises means (7) responsive to the applied pressure difference to produce a signal (8) which is utilised to provide feedback to the pressure difference applying means (9), to maintain a predetermined constant pressure difference.

8. Apparatus according to claim 6 or 7, in which the microsize tunnel or tunnels extend into one or more crystalline substrates (1,2).

9. Apparatus according to claim 6, claim 7 or claim 8, in which there are several straight, parallel tunnels of equal length.

10. Apparatus according to claim 6, claim 7, claim 8 or claim 9, in which the tunnel, or at least one of the tunnels

has at least two portions with different cross-sections, for comparing the behaviour of the cells passing through such portions.

11. A pump (9) for delivering predetermined volumes of fluid comprising a deformable fluid reservoir (22) constrained from isovolumetric deformation, and characterised by a piezoelectric drive element (26) adjacent the reservoir for deforming the reservoir, in response to an electric drive potential (30), in a time-continuous manner, smoothly to change its volume thereby to expel fluid therefrom.

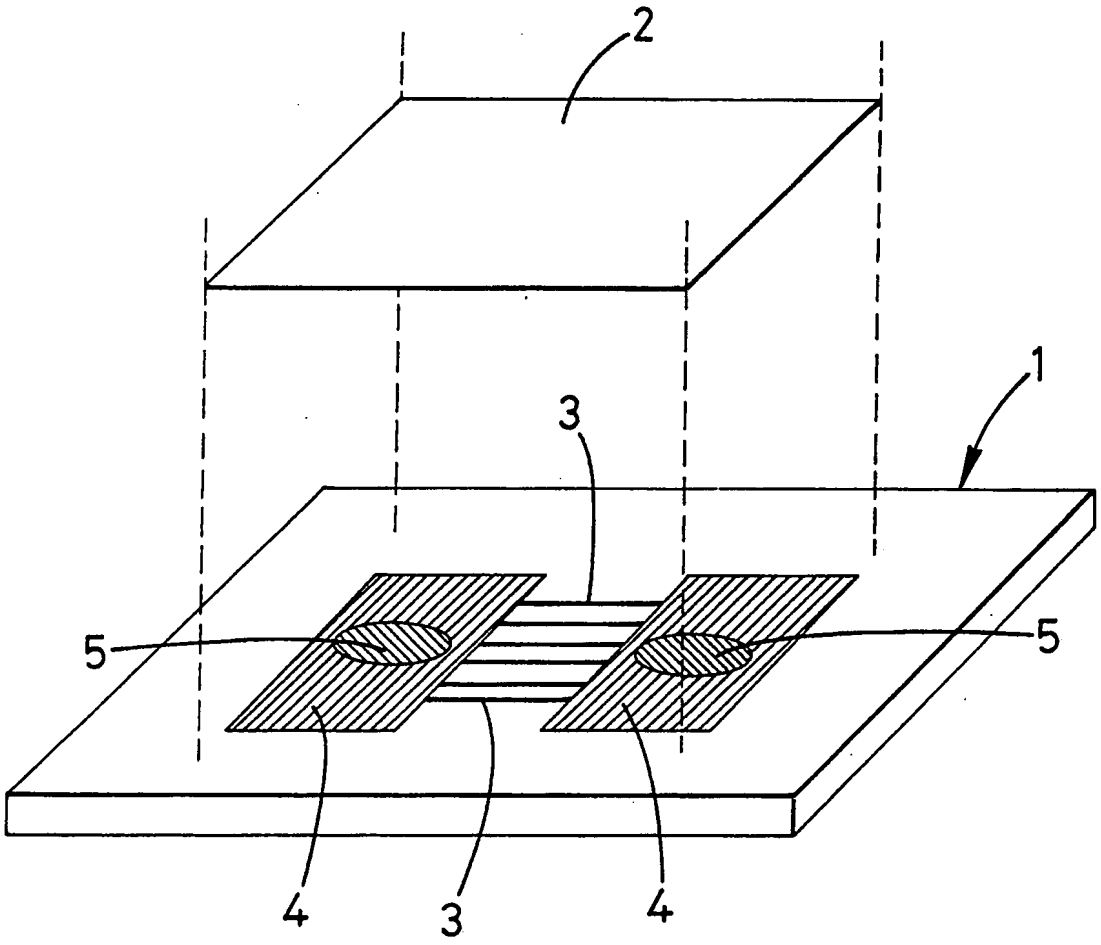

FIG. 1

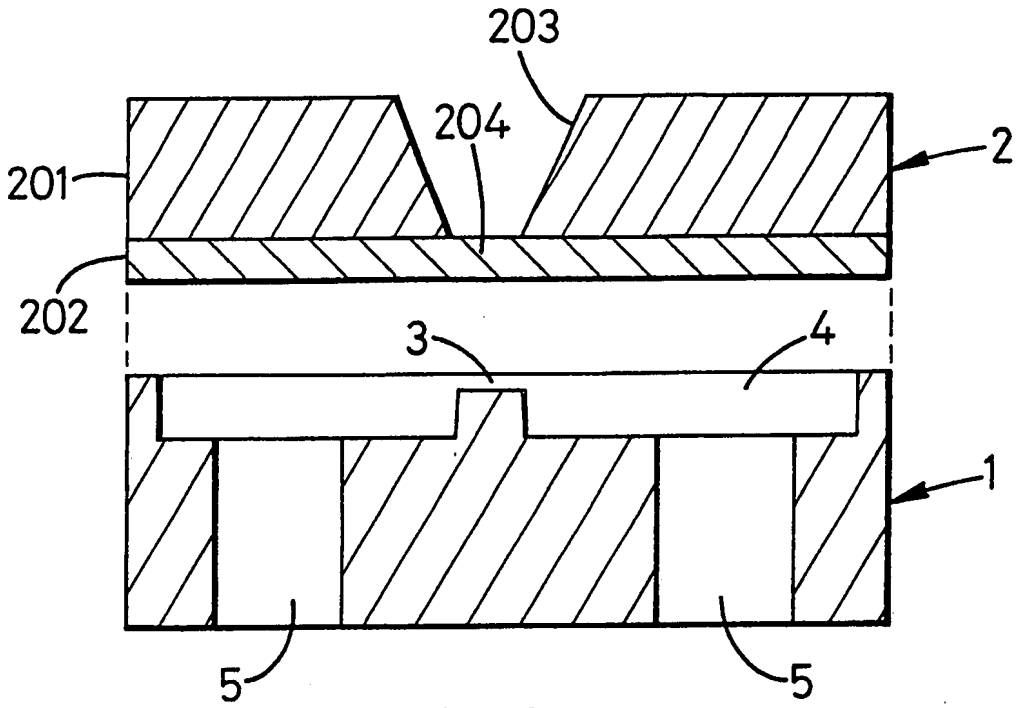

FIG. 2

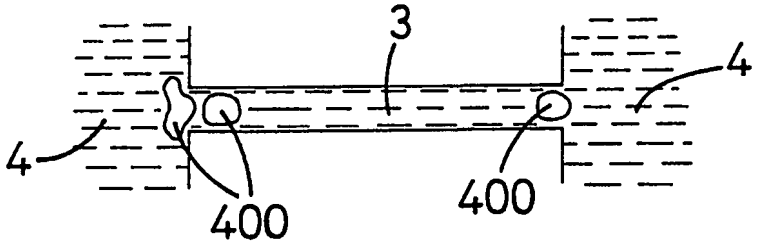

FIG. 4

FIG.3

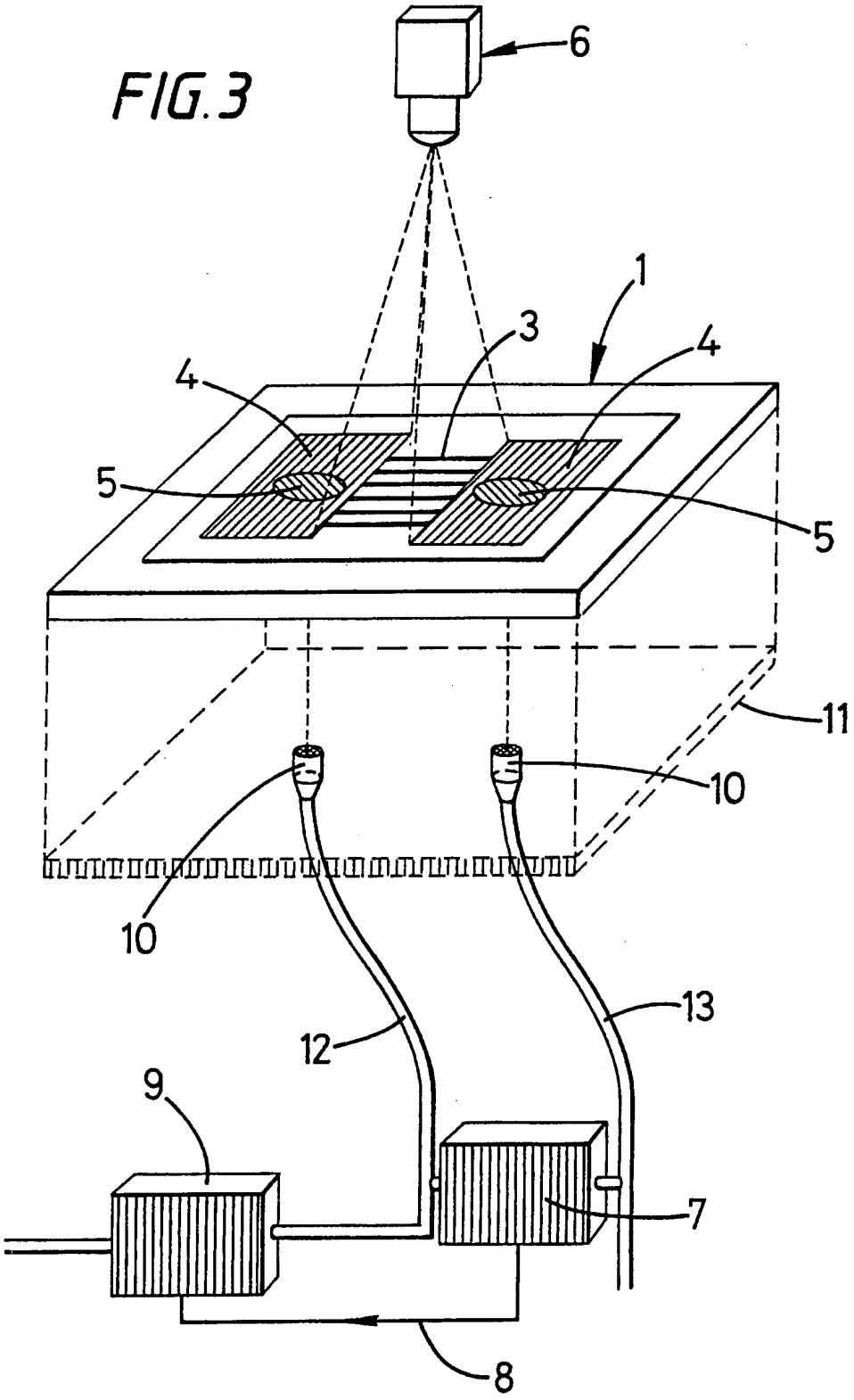

FIG. 5